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<b>(21) International Application Number:</b> PCT/AU98/00313 <b>(22) International Filing Date:</b> 1 May 1998 (01.05.98) <b>(30) Priority Data:</b> PO 6568 1 May 1997 (01.05.97) AU PP 0814 8 December 1997 (08.12.97) AU <b>(71) Applicant (for all designated States except US):</b> NOVOGEN INC. [US/US]; Corporate Agents Inc, 1013 Centre Road, Wilmington, DE 19805 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> KELLY, Graham, Edmund [AU/AU]; 1,47 Coolawin Road, Northbridge, NSW 2063 (AU). <b>(74) Agents:</b> STEARNE, Peter, Andrew et al.; Davies Collison Cave, Level 10, 10 Barrack Street, Sydney, NSW 2000 (AU).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> TREATMENT OR PREVENTION OF MENOPAUSAL SYMPTOMS AND OSTEOPOROSIS  <b>(57) Abstract</b>  There is described a method for the treatment or prevention of menopausal symptoms or osteoporosis wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone formononetin, or a method for the treatment or prevention of menopausal symptoms wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone daidzein, the isoflavone being optionally administered with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients. Therapeutic uses and compositions/foods are also described, comprising daidzein or formononetin optionally in association with one or more pharmaceutically acceptable adjuvants, carriers, food components and/or excipients.		

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## TREATMENT OR PREVENTION OF MENOPAUSAL SYMPTOMS AND OSTEOPOROSIS

This invention relates to compositions, therapeutic uses and methods of treatment or  
5 prevention of menopausal symptoms and osteoporosis.

Menopausal symptoms and osteoporosis are significant scourges in the female population,  
generally affecting many women in later life.

10 Menopausal symptoms are very well known and are described, for example, by Greene, J.G.  
and Cooke, D.J. (1980) *British Journal of Psychiatry* Volume 136, 486-491 (incorporated  
herein by reference). Hot flushes are one of the principal menopausal symptoms which are  
uncomfortable and irritating. Greene and Cooke have developed a score in order to measure  
menopausal symptoms in women. This score is approved by the US Department of Health  
15 and widely used in the medical community. The indicators of menopausal symptoms  
according to Greene and Cooke comprise hot flushes, sweating at night, heart beating quickly  
or strongly, feelings of tension or nervousness, difficulty in sleeping, excitability, attacks of  
panic, difficulties in concentrating, feelings of tiredness or lack of energy, unhappiness or  
depression, crying spells, irritability, feelings of dizziness or faintness, pressure or tightness  
20 in head or body, parts of the body feeling numb or tingling, dry vagina and/or dry mouth,  
headaches, muscle and joint pains, loss of feeling in hands or feet, breathing difficulties, and  
loss of interest in sex.

The peri-menopausal stage of life in women is associated with a fall in blood levels of the  
25 three major estrogens - estradiol, estrone and estriol - which occurs naturally in women  
usually between 45 - 55 years of age. The primary or acute menopause symptoms which  
effect menopausal women include hot flushes and night sweats. These are associated with  
often dramatically increased blood perfusion of the skin producing discomfort and sweating.

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The precise mechanism of these symptoms is unknown but generally is thought to represent disturbance to normal homeostatic mechanisms controlling vasomotor activity and thermoregulation.

- 5 The fact that treatment and/or prevention with replacement estrogens usually relieves the symptoms (so-called estrogen replacement therapies) establishes the link between these symptoms and an estrogen deficiency. The menopausal stage of life is associated with a wide range of other acute symptoms as described above and these symptoms are generally estrogen-responsive.

10

- Osteoporosis is believed to affect one third to one half of all post-menopausal women. In the United States it has been reported that annually 500,000 bone fractures occur as a result of osteoporosis. It is further reported that nearly one third of women over 65 will suffer at least one bone fracture resulting from osteoporotic bone weakening. Increased calcium intake and  
15 other approaches are suggested to have some effect. However, the widespread effects of osteoporosis indicates effective approaches for prevention/treatment have not yet arisen.

- It has previously been thought that reduction in endogenous estrogen levels which occurs prior to menopause causes or contributes to the symptoms of menopause, as well as post-  
20 menopausal osteoporosis.

Isoflavones, being plant chemicals which occur largely in members of *Leguminosae*, display a range of biological functions which have suggested they may be useful in treating a host of medical conditions.

25

- A small sub-group of isoflavones (comprising daidzein, genistein, biochanin, and formononetin and) is distinguished by their ability to bind to estrogen receptors on animal (including human) cells. This is due to the close similarity of the steric structure of the diphenolic rings of isoflavones with the steroidal ring structure of estrogens such as estradiol,  
30 estrone and estriol. Although having substantially lower binding affinity to the receptor compared to steroidal estrogens, estrogenic isoflavones are weakly estrogenic. This group

of five isoflavones have the most basic diphenolic structure possible in contrast to the relatively more complex structures of other isoflavonoid compounds. This simplicity of structure and its close proximity in shape to the steroidal ring structure of estrogenic hormones is believed to grant these compounds their estrogenicity. This group also exhibits a range of biological functions in animal cells which appear to be independent of the estrogen receptor and these include anti-oxidant, diuretic, anti-spasmodic and anti-cancer effects. These interesting functions with their potential therapeutic benefits has brought this particular group of isoflavones to the attention of medical researchers in recent years.

10 In the plant, the isoflavones can occur in a variety of forms - (i) in the basic form, (ii) in a malonyl form, and (iii) in an acetyl form; the isoflavones are biologically active in each of these forms. The naturally-occurring state for each of these forms is as a glycoside, being bound to a sugar moiety such as glucose to produce a water-soluble form. In this form, the isoflavone has enhanced stability to degradative factors such as heat, oxidation and ultraviolet  
15 irradiation. This water-soluble form also permits transport of the isoflavone both around the plant and intra-cellularly. At the intra-cellular site of biochemical function of the isoflavone, an intra-cellular glucosidase enzyme cleaves the sugar moiety, leaving the more biologically active, but water-insoluble, aglucone form.

20 When ingested in the diet, the isoflavones undergo varying degrees of metabolism within the gut, within the gut wall, and within the liver before entering the parenteral bloodstream to exert their biological effects. The first metabolic process is the hydrolysis of the glucosidic form to the aglucone form. This occurs as a result both of low pH from gastric acid and of the action of  $\beta$ -glycosidase enzyme activity within bowel bacteria.

25

Some of the aglucone isoflavones are absorbed intact and in passing through the gut wall are believed to be glucuronated or sulphonated as per steroidal compounds. The bulk of isoflavones are fermented within colonic bacteria. One of the fermentation processes is to demethylate isoflavones (eg. formononetin gives daidzein and biochanin gives genistein on  
30 demethylation). In another series of fermentation steps, daidzein and genistein are converted to a range of end-products including equol, dehydroequol, O-desmethylangolensin (ODMA),

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6-hydroxy-ODMA, 2-dehydro-ODMA, dihydrodaidzein, tetra-hydrodaidzein and dihydrogenistein. The liver is capable of further demethylation of isoflavones such as formononetin and biochanin to the more basic daidzein and genistein structures. The isoflavones and their metabolites and derivatives circulate freely within the body and are  
5 excreted primarily in the urine with smaller amounts in the faeces.

The possibility that dietary estrogenic isoflavones may have some therapeutic benefit in acute menopausal symptoms was suggested by the observation that Japanese women who typically have much higher dietary levels of isoflavones (mostly derived from soya) compared to  
10 women in Western countries have a reportedly lower incidence of acute menopausal syndrome symptoms such as hot flushes. This has led to somewhat speculative claims of therapeutic benefit of the isoflavones from the group daidzein, formononetin, biochanin and genistein in the treatment and/or prevention of acute menopausal syndrome symptoms (US patent 5,498,631 - Gorbach *et al*).

15

Gorbach analysed urinary isoflavone excretion in Japanese subjects who consumed a traditional Japanese low-fat diet. The presence of estrogenic isoflavones in the urine of the women, men and children studied suggested to Gorbach that the isoflavones produced a therapeutic effect. The obvious flaws in this study, namely that in a diet with high isoflavone  
20 intake significant urinary out-put of isoflavones would be expected, and the huge number of biochemically active species in any diet make it impossible to ascribe biological effects to any particular component or components indicate that Gorbach's claims do not stand scrutiny. The fact that a community with a particular health profile happens to have a high dietary level (and high body levels) of a certain plant component in no way establishes cause and effect.  
25 This is only achieved through appropriately conducted clinical studies where well accepted scientific principles can be applied.

Clinical and other studies done to date in this area are highly equivocal, with no consistent effect reported. Reported studies have involved the challenge of peri-menopausal women  
30 either with whole foodstuffs (such as soyflour) containing isoflavones or with extracts of soya or other legumes, often together with other agents such as vitamins, or isoflavones together

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with estrogen and/or vitamins and various minerals. It is to be noted that soy does not contain the isoflavones formononetin and biochanin. Even when a positive clinical effect has been obtained, it has been with a mixture of a plurality of isoflavones, as well as a wide range of other unidentified dietary components and other biologically active components - it is known for example that other compounds present in legumes such as flavonoids (eg. quercetin, luteolin, kaempferol and lignans) also are estrogenic and it is also likely that among the other 700 or so isoflavonoids present in the *Leguminosae* family there are as yet unidentified isoflavonoids with estrogenic activity.

10 Gorbach suggests that isoflavanoids bind to estrogen receptors, exerting an estrogenic effect in menopausal women. However, it is acknowledged generally that no direct evidence exists to link the presence of isoflavones with a therapeutic effect in treatment and/or prevention of acute menopausal syndrome symptoms. But even if such a link could be inferred from the current epidemiological and clinical studies, which it can not, the question remains what, if  
15 any, therapeutic effect is the result of a collective effect of the estrogenic isoflavones - daidzein, formononetin, biochanin, genistein. All four isoflavones are estrogenic, but they have quite different estrogenic potencies. The relative estrogenicity of genistein, daidzein, formononetin, biochanin is 1.3, 0.09, 0.01, 0.07 respectively (relative to 17 $\beta$ -estradiol 100). Hence formononetin and biochanin have negligible estrogenic activity. On this basis, and  
20 given the relative proportions of daidzein and genistein in the blood of Japanese maintaining a typical Japanese diet, it might be inferred that genistein potentially is the most potent isoflavone as far as acute menopause syndrome symptoms are concerned.

Estrogenic isoflavones have also been identified as possible therapeutic compounds in the  
25 treatment and/or prevention of osteoporosis. Asian populations consuming large amounts of phytoestrogen-rich soybeans and vegetables appear to be protected to a greater extent than western populations from the problems associated with osteoporosis. These observations are by no means clear, and are contradicted in a number of studies.

30 Fujita and Fukase (Proc. Soc. Exp. Biol. Med. 200(2) 149-5, 1992) indicates that in osteoporosis analysis between Japanese and US populations diet is not of particular

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significance, with bone mass being very similar in both populations. Instead they suggest the outcomes of osteoporosis are more likely associated with lifestyle affecting muscle development and motor control. Arjanandi *et al* (American Institute of Nutrition, p161-167, 1995) indicates that any protective effects of soy on bone is associated with soy protein. Hunt  
5 *et al* (Am. J. Clin. Nutr., p 517-523, 1989) indicate that there is no appreciable difference in bone density between elderly menopausal omnivores, and elderly menopausal vegetarians whose diet included isoflavone rich plant materials.

Published European Patent Application No. 0135172 (Takeda, published 27 May 1985)  
10 discloses methods for the treatment of osteoporosis by administration of 7,4-dihydroxy isoflavone (daidzein) relying on its estrogenic activity. This finding is inconsistent with the biological studies reported above. Moreover, Tobe *et al* (Biosci. Biotech. Biochem. 61(2) 370-371, 1997) show that daidzein stimulates bone resorption, that is bone breakdown, and would be contra-indicated for treating osteoporosis, as it would worsen an existing condition,  
15 and possibly pre-dispose a non affected person to osteoporosis.

Against the foregoing background, the present invention is predicated upon our surprising finding that, within the framework of what was conjectured regarding the treatment/prevention of menopausal symptoms and osteoporosis, formononetin may be used  
20 to treat both menopausal symptoms and osteoporosis, and daidzein may be used in the treatment of menopausal symptoms. Our findings indicate that formononetin has pronounced clinical activity in the treatment and/or prevention of menopausal symptoms and in the treatment and/or prevention of osteoporosis as does daidzein in the treatment/prevention of menopausal symptoms. This is highly unexpected given the negligible estrogenic effect of  
25 formononetin, the bone resorption activity of daidzein and the established view that formononetin was very rapidly metabolised to daidzein in the gut.

### Summary of the Invention

30 There is provided in a first aspect of this invention a method for the treatment or prevention of menopausal symptoms or osteoporosis wherein there is administered to a subject in need



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of such treatment a therapeutically effective amount of the isoflavone formononetin, or a method for the treatment or prevention of menopausal symptoms wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone daidzein, the isoflavone being optionally administered with one or more  
5 pharmaceutically acceptable adjuvants, carriers and/or excipients.

In another aspect of the invention there is provided a pharmaceutical composition for the treatment or prevention of menopausal symptoms or osteoporosis wherein the said composition comprises the isoflavone formononetin or a pharmaceutical composition for the  
10 treatment or prevention of menopause wherein said composition comprises the isoflavone daidzein together with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

In another aspect of the invention there is provided use of the isoflavone formononetin in the  
15 treatment or prevention of menopausal symptoms or osteoporosis, or use of the isoflavone daidzein in the treatment or prevention of menopausal symptoms, the isoflavone being optionally administered with one or more pharmaceutically acceptable adjuvants, carriers, and/or excipients.

20 In a further aspect of the invention there is provided an agent for the treatment or prevention of menopausal symptoms or osteoporosis, or an agent for the treatment or prevention of menopausal symptoms which comprises daidzein optionally in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

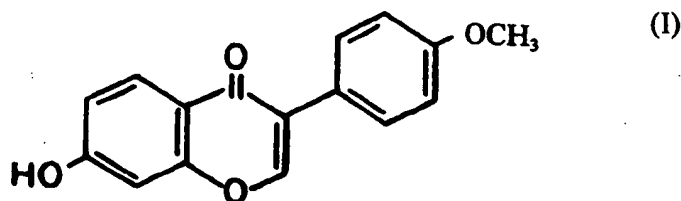
## 25 Detailed Description

Throughout this specification and the appended claims, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" or "include" or "including", will be understood to imply the inclusion of a stated element or integer or group  
30 of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The present invention provides in a first aspect a method for the treatment or prevention of menopausal symptoms or osteoporosis wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone formononetin, or a method for the treatment or prevention of menopausal symptoms wherein there is administered to a  
5 subject in need of such treatment a therapeutically effective amount of the isoflavone daidzein, the isoflavone being optionally administered with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

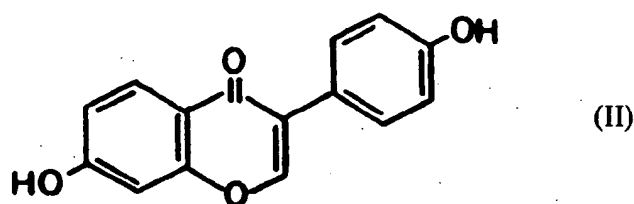
It is believed that the invention described represents a substantial breakthrough in the field  
10 of treatment and/or prevention of menopausal symptoms and osteoporosis. The administration to subjects of plant extracts containing a range of isoflavones may be unpleasant in the sense that the extract may not be particularly palatable, and/or may contain a host of ill-defined compounds which may affect disadvantageous biological activity. Wilcox et al (*British Medical Journal* (1990) 301: 905) have reported increases in vaginal cell  
15 proliferation amongst post-menopausal women consuming soybean phytoestrogens for six weeks. In addition Markiewicz et al (*J. Steroid Biochem.* (1993) 45: 399) have shown experimentally that the soy isoflavone genistein exhibited an estrogen effect on endometrial cancer cells, that is, potentiated cancer cell growth in this cell type. Such reports raise questions about the safety of comparatively high doses of genistein. The present invention  
20 provides the treatment or prevention of menopausal symptoms and osteoporosis without any side effects caused by uncharacterised biologically active plant materials (such as coumestrols), or other disadvantageous effects.

The menopausal symptoms which may be treated according to the method of this invention  
25 are those described by Greene, J.G. and Cooke, D.J. (1980) *British Journal of Psychiatry* Volume 136, 486-491. Preferably the menopausal symptoms treated or prevented according to the present invention are hot sweats and night time sweats, more particularly hot sweats. Having said this, the method of the invention is applicable to the treatment and/or prevention of other symptoms of menopause as previously described. The isoflavone formononetin is  
30 of the formula (I):



Although it was previously thought that formononetin was almost immediately metabolised (demethylated) to daidzein upon administration to a subject, the present inventors have found that formononetin persists in the blood stream for a considerable time (having a half life of generally about 20 hours).

10 The isoflavone daidzein is of the formula (II):



20 Formononetin or daidzein are preferably administered to a subject substantially unaccompanied by other isoflavones. By this is meant that any composition or preparations may contain minor amounts of other isoflavones, in the order of 10% (w/w) or less. Preferably the formononetin or daidzein represents at least 90% of isoflavone content, more preferably 95%, even more preferably 98% or more. Genistein, if present, is in amounts of  
25 about 5% or less, more preferably less than 1% (w/w) with regard to isoflavone content. It is recognised by regulatory agencies that an isoflavone content in the order of 95% of total isoflavones represents effective purity.

In the treatment of menopausal symptoms formononetin may be administered in combination  
30 with daidzein, for example from a ratio of 1:10 to 10:1.

Daidzein metabolites may be used in place of daidzein in the various embodiments of this invention. These metabolites include equol, o-desmethyldangolensin (ODMA), dehydroequol, 2-dehydro-ODMA, 6-hydroxy-ODMA, dihydrodaidzein and tetra-hydrodaidzein (which collectively may be referred to hereinafter as daidzein metabolites). Accordingly, in a further  
5 aspect this invention extends to a method for the treatment or prevention of menopausal symptoms or osteoporosis wherein there is administered, to a subject in need of such treatment and/or prevention, formononetin or a daidzein metabolite, optionally administered with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients. The formononetin or daidzein metabolites may be administered in a form substantially  
10 unaccompanied by other isoflavones.

Daidzein and/or formononetin compositions or preparations are administered in an amount, and under a dosage regime which gives relief to menopausal symptoms or osteoporosis. With regard to menopausal symptoms this can be readily determined by the subject who is being  
15 treated, or by their physician. Generally, it is found that prevention or therapy of menopausal symptoms and osteoporosis results from daily administration of formononetin such as from one to six times in a 24 hour period, as does the treatment or prevention of menopausal symptoms with daidzein, so as to give a daily dose of the isoflavone in an amount from about 5 mg to about 400 mg per day (this dosage range may be referred to as the  
20 "effective amount").

Formononetin and daidzein may be prepared by synthesising the compounds by conventional chemical synthetic techniques as are well known in the art, or by purification from extracts of plants of the genus *Leguminosae*, particularly from soy (such as from soy flour, soy  
25 hypocotyls) and clover (such as red clover, and subterranean clover) such as to form a formononetin or daidzein composition or preparation.

Compositions/preparations administered to subjects for the treating and/or prevention of, or for reducing the predisposition to, menopausal symptoms or osteoporosis may comprise in  
30 addition to the specific isoflavones previously mentioned formononetin optionally administered with one or more pharmaceutically acceptable adjuvants, carriers and/or

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excipients, so as to form a composition or preparation. Pharmaceutically acceptable, adjuvants, carriers and/or excipients, and the like, are well known in the art, for example as described in the *Handbook of Pharmaceutical Excipients*, second edition, American Pharmaceutical Association, 1994 (incorporated herein by reference). Daidzein or  
5 formononetin may be administered in the form of tablets, capsules, powders for reconstitution, syrups, foods (such as food bars, biscuits, snack foods and other standard food forms well known in the art) or in drink formulations. Drinks may contain flavouring, buffers and the like.

- 10 In the method of this invention calcium may be co-administered (that is before at the same time or after the isoflavones previously mentioned), for example as a separate tablet, or as part of a suitable dosage form.

In a further aspect of this invention there is provided a pharmaceutical composition for the  
15 treatment or prevention of menopausal symptoms or osteoporosis wherein the said composition comprises the isoflavone formononetin or a pharmaceutical composition for the treatment or prevention of menopause wherein said composition comprises the isoflavone daidzein, together with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients. As mentioned above, pharmaceutically acceptable adjuvants, carriers and/or  
20 excipients are well known in the art. Examples include compositions according to the present invention may include one or more pharmaceutically acceptable carriers. The carriers are selected so as to be acceptable in the sense of being ingredients in the composition and must not be deleterious to the patient. The carriers may be solid or a liquid, or both, and may be formulated with the extract as a unit-dose, for example a tablet, which may contain from  
25 0.5% to 59% by weight of the active compound or up to 100% by weight to the active compound. Compositions may be prepared by any of the well known techniques of pharmacy, for example admixing the components, optionally including excipients, diluents (for example water) and auxiliaries as are well known in the pharmaceutical field.

- 30 The compositions of the invention include those suitable for oral, rectal, optical, buccal (for example sublingual), parental (for example subcutaneous, intramuscular, intradermal and

intravenous) and transdermal administration. The most suitable route in any given case will depend on the nature and severity of the condition being treated and the state of the patient.

Compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the extract; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active isoflavone and one or more suitable carriers (which may contain one or more accessory ingredients as noted above). In general the compositions of the invention are prepared by uniformly and intimately admixing the isoflavone with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by comprising or moulding a powder or granules containing the extract, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the extracts in the form of a powder or granules optionally mixed with a binder, lubricant, inert diluents, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Suitable carriers may be fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and also binders, such as starch pastes using, for example, corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose and/or polyvinylpyrrolidone, and, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, cross linked polyvinyl pyrrolidone, agar or alginic acid or a salt thereof, such as sodium alginate. Excipients may be flow conditioners and lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Dragée cores are provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric

coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Dyes or pigments may be added to the tablets or dragée coatings, for example for identification purposes or to indicate different doses of active ingredients.

5

Other orally administrable pharmaceutical compositions are dry-filled capsules made, for example, of gelatin, and soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The dry-filled capsules may comprise the extracts in the form of granules, for example in admixture with fillers, such as lactose, binders, such as starches,  
10 and/or glicants, such as talc or magnesium stearate, and, where appropriate, stabilisers. In soft capsules, the extract is preferably dissolved or suspended in suitable liquids, such as fatty oils, paraffin oil or liquid polyethylene glycols, to which stabilisers may also be added.

Formulations suitable for buccal (sublingual) administration include lozenges comprising the  
15 extracts in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the isoflavones with one or more  
20 conventional solid carriers, for example cocoa butter, and then shaping the resulting mixture.

Compositions may include calcium or other active agents suggested to provide some amelioration of osteoporosis or symptoms of menopause.

25 Pharmaceutical compositions generally comprise from about 5 mg to about 400 mg of the isoflavone/s and may be administered one or more times per day.

In a further aspect of this invention there is provided use of the isoflavone formononetin in the treatment or prevention of menopausal symptoms or osteoporosis, or use of the isoflavone  
30 daidzein in the treatment or prevention of menopausal symptoms, the isoflavone being

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optionally administered with one or more pharmaceutically acceptable adjuvants, carriers, and/or excipients.

In another aspect of the invention there is provided use of formononetin, for the manufacture  
5 of a medicament for the treatment or prevention of menopausal symptoms or osteoporosis, or use of daidzein or formononetin for the manufacture of a medicament for the treatment or prevention of osteoporosis. Generally, the isoflavone is provided in the form of a medicament in association with one or more pharmaceutically adjuvants, carriers and/or excipients. Daidzein and/or formononetin may be conveniently blended as a dry powder with  
10 other components and formed into appropriate dosage forms. Such medicaments generally comprise from about 5 mg to about 400 mg of isoflavone.

According to a still further aspect of the invention there is provided an agent for the treatment or prevention of menopausal symptoms or osteoporosis, or an agent for the treatment or  
15 prevention of menopausal symptoms which comprises daidzein optionally in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

Daidzein and/or formononetin may be administered in the form of a dietary product, as mentioned above, for example in a palatable food carrier such as a confectionary bar, biscuit,  
20 cereal or beverage.

The methods and compositions of the present invention do not include the use of estrogens, or components such as licorice, cholecalciferol and vitamin E. Estrogen administration has many side effects such as genital bleeding and hepatic disorders. Licorice has vasoactive  
25 activity and may exacerbate menopause symptoms or osteoporosis. Cholecalciferol and vitamin E are also disadvantageous.

The foregoing description in relation to daidzein, applies to the daidzein metabolites equol, o-desmethylangolensin (ODMA), dehydroequol, 2-dehydro-ODMA, 6-hydroxy-ODMA,  
30 dihydrodaidzein and tetra-hydrodaidzein, as well as combinations of the above species.



Hence, daidzein may be replaced by one or more of these metabolites. Daidzein or formononetin may also take the form of aglycones, glycosides, malonyl or acetyl derivatives.

This invention will now be described with reference to the following non-limiting examples.

5

#### Example 1

Peri-menopausal women with symptoms of acute menopausal syndrome including at least an average of 3 hot flush episodes per day were treated with a preparation containing a  
10 concentrated amount of daidzein, formononetin, genistein and biochanin prepared according to the method described in published Australian patent application 40523/93 (incorporated herein by reference). The preparation contained 100 mg isoflavones containing biochanin and formononetin (in the ratio of 1.8:1). Urine samples were collected from each woman at the start of the test and then again at the end of the trial and the levels of particular isoflavones  
15 in the urine (as a marker of total body isoflavone ratios) then correlated with the degree of reduction in the incidence of hot flushes per day over the course of the study. Daidzein and genistein were detected in the urine of all test subjects but the levels were highly variable, ranging from barely detectable to relatively high. Formononetin and biochanin levels ranged between undetectable and significant. Genistein levels showed no correlation with therapeutic  
20 response; daidzein levels correlated well with therapeutic response indicating potential therapeutic utility of daidzein and formononetin.

#### Example 2

25 Daidzein substantially free of other isoflavones was prepared from soy by the following method. 1 kg of defatted soyflour (readily available from many commercial sources) was added to 10 L of water containing 50 g of glucan hydrolase enzyme (Bio-Feed Beta CT, Novo Nordisk, Denmark). To this suspension 5 L of ethyl acetate is then added. This mixture is mixed vigorously using a high pressure pump for 3 hours so that it forms an emulsion. This  
30 mixing ensures effective contact between the aqueous phase and the miscelles of ethyl acetate so that the enzymatically hydrolysed aglucone forms of the isoflavones move from the

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- aqueous to the organic solvent phase. The three phases (soyflour, aqueous, ethyl acetate) are separated by centrifugation in a swing bucket centrifuge at 2000 g for 30 minutes. The upper phase comprising ethyl acetate is aspirated, 200 ml of water added, and then placed into a rotary evaporator at 75°C under a weak vacuum. Upon removal of the ethyl acetate, the
- 5 residual aqueous phase containing the isoflavones is extracted once with 500 ml of hexane to remove oils and fats, and then twice with 500 ml of octanol which selectively removes genistein. The aqueous phase then was taken to dryness overnight in an oven at 80°C. This material was shown by high pressure liquid chromatographic analysis to comprise 65% isoflavones comprising daidzein (95%) : genistein(5%) : formononetin(0%) : biochanin(0%).
- 10 This material was mixed with standard carriers/excipients and tableted to give 500 mg tablets containing 25 mg daidzein. In this particular example, the daidzein was tableted with equivalent (w/w) amounts of microcrystalline cellulose, calcium hydrogen phosphate, magnesium stearate and anhydrous colloidal silica.
- 15 Formononetin substantially free of other isoflavones was prepared from clover by enzyme/solvent as above. Formononetin was recovered as a purity level greater than 95% by chromatographic analysis or by HPLC.

### Example 3

20

- The dried end product of the first part of Example 2 above can be used to further concentrate genistein or daidzein with/without. 3 kg of this material is mixed with 1000 L of an organic solvent such as acetone, chloroform or octanone, but preferably acetone for reasons of safety and cost. Each of these 3 solvents has been shown by the inventors to have high affinity for
- 25 genistein but not daidzein. The mixture is stirred continuously at room temperature for between 1-24 hours but preferably 2 hours during which time a large amount (approximately 75%) of the genistein transfers into the solvent phase. The mixture is allowed to settle for about 2 hours, the solvent is separated from the residue (Sample 2) and transferred to a still for evaporation. Sample 2 material preferably is extracted with acetone a further 1-5 times
- 30 (preferably 4 times). This material typically contains 76% daidzein, 1% genistein, 0.5% glycitein, with the remainder comprising residual lipid soluble material such as short chain

fatty acids. On a w/w basis compared with the other isoflavones, this preparation contains 98% (w/w) daidzein. Daidzein may be purified to 95% (w/w) of total material or more purity by preparative purification regimens such as preparative HPLC. A daidzein preparation according to this example is tableted with conventional inert excipients according to Example 3 to give a 200 mg tablet containing 50 mg daidzein.

#### Example 4

A group of 36 post-menopausal women experiencing menopausal symptoms were treated with a composition containing either 15 mg daidzein or 60 mg daidzein administered on a daily basis for 3 months. Compared to a placebo control group there was a significant decrease in the Greene Score for menopausal symptoms which corresponds to treatment/amelioration of menopausal symptoms (such as hot flushes). The urinary profile of these subjects demonstrated the therapeutic effectiveness of daidzein as against other isoflavones.

15

In a similar study the same dosage levels of formononetin again gave a significant decrease in Greene Score for menopausal symptoms as for daidzein.

#### Example 5

20

A pharmacokinetic study involving 16 human patients aged between 18 and 40 was conducted, to determine the pharmacokinetics of formononetin following oral administration. Each patient was orally administered 9.3mg of formononetin with 200 ml of purified water and maintained a low isoflavone diet for one week prior to, and during the study. Blood samples were taken at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8, 10 and 12 and 24 hours post administration, and analysed for formononetin concentration.

An analysis of formononetin concentrations (ng/ml) against time demonstrated that contrary to previous opinion, unmetabolised formononetin persists in the blood stream for considerable time following administration (having a half life of about 20 hours).

30

**Example 6**

15 post-menopausal women who are experiencing menopausal systems are each administered 60 mg formononetin daily for three months. Relative to a control group the treatment group  
5 show a significant decrease in Greene Score for menopausal symptoms.

A similar study involving 10 post-menopausal women in a high risk group for osteoporosis were also administered 60 mg formononetin daily for three months. Preliminary results indicate protection against osteoporosis in the treatment group when measured by bone  
10 density and bone turnover markers were measured.

**Example 7**

The second study (double-blind, placebo-controlled) involved a six month study of the effects  
15 of phytoestrogen formononetin on bone resorption markers in twenty post-menopausal women. The aim of the study was to evaluate the efficacy of a defined daily quantity of isoflavone at 25 mg, 50 mg or 75 mg on bone resorption makers in post menopausal women and compare these to normal controls. The effects of isoflavones on endometrial thickness, circulatory lipids and coagulation factors were also evaluated. Subjects were also given  
20 supplemental calcium in a dose of 1200 mg per day.

Bone density measurements using a bone densitometer were made at three sites of the forearm at 0.3 and 6 months and showed significant improvement in bone density. Bone markers for osteocalcin, deoxy pyridinoline crosslinks, N-terminal collagen crosslinks, calcium and other  
25 markers showed a similar improvement in bone resorption and turnover.

It is to be recognized that the present invention has been described by way of example only, and that various modifications and/or alterations which would be obvious to a person skilled in the art, on the basis of the teaching herein, can be made thereto without departing from  
30 the intended scope or spirit of the invention.

**CLAIMS**

1. A method for the treatment or prevention of menopausal symptoms or osteoporosis wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone formononetin, or a method for the treatment or prevention of menopausal symptoms wherein there is administered to a subject in need of such treatment a therapeutically effective amount of the isoflavone daidzein, the isoflavone being optionally administered with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.
2. A method according to claim 1 for the treatment or prevention of osteoporosis.
3. A method according to claim 2 wherein formononetin is administered to a subject.
4. A method according to claim 1 for the treatment or prevention of menopausal symptoms.
5. A method according to claim 4 wherein formononetin or daidzein is administered to a patient.
6. A method according to claim 1, wherein the isoflavone is present in an amount of at least 90% (w/w) as against any other isoflavones.
7. A method according to claim 1, wherein the isoflavone is administered at least once per day.
8. A method according to claim 1, wherein the isoflavone is administered in an amount from about 5 mg/day through to about 400 mg/day.
9. A method according to claim 1, wherein the isoflavone is administered from 1 to 6 times in a 24 hour period so as to give a daily dosage of from about 5 to about 400 mg.

- 20 -

10. A method according to claim 1 wherein said isoflavone is derived from a plant extract comprising daidzein and/or formononetin substantially unaccompanied by other isoflavones.
11. A method according to claim 1 for the treatment or prevention of osteoporosis wherein a combination of formononetin and daidzein is administered to a patient.
12. A method according to claim 1 wherein the isoflavone is substantially unaccompanied by other isoflavones.
13. A method according to claim 1 wherein daidzein is replaced by a daidzein metabolite selected from equol, o-desmethyldangolensin (ODMA), dehydroequol, 2-dehydro-ODMA, 6-hydroxy-ODMA, dihydrodaidzein and tetra-hydrodaidzein.
14. A pharmaceutical composition for the treatment or prevention of menopausal symptoms or osteoporosis wherein the said composition comprises the isoflavone formononetin or a pharmaceutical composition for the treatment or prevention of menopause wherein said composition comprises the isoflavone daidzein, together with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.
15. A composition according to claim 14, which is a composition for the treatment or prevention of osteoporosis.
16. A composition according to claim 15 which includes the isoflavone formononetin.
17. A composition according to claim 14 which is a composition for the treatment and/or prevention of acute menopausal symptoms.
18. A composition according to claim 17 wherein the isoflavone is daidzein or formononetin.

19. A composition according to claim 17 which includes a combination of daidzein and formononetin.
20. A composition according to claim 14 which comprises from 5 to 400 mg of isoflavone.
21. A composition according to claim 14 wherein daidzein is replaced by a daidzein metabolite selected from equol, o-desmethylangolensin (ODMA), dehydroequol, 2-dehydro-ODMA, 6-hydroxy-ODMA, dihydrodaidzein and tetra-hydrodaidzein.
22. Use of the isoflavone formononetin in the treatment or prevention of menopausal symptoms or osteoporosis, or use of the isoflavone daidzein in the treatment or prevention of menopausal symptoms, the isoflavone being optionally administered with one or more pharmaceutically acceptable adjuvants, carriers, and/or excipients.
23. Use according to claim 22, wherein the isoflavone is administered in an amount from about 5 mg to about 400 mg.
24. Use according to claim 22 for the treatment or prevention of osteoporosis wherein the isoflavone is formononetin.
25. Use according to claim 22 for the treatment or menopausal symptoms wherein the isoflavone is formononetin or daidzein.
26. Use according to claim 25 wherein a combination of daidzein and formononetin are administered to a subject.
27. Use of formononetin, for the manufacture of a medicament for the treatment or prevention of menopausal symptoms or osteoporosis, or use of daidzein or formononetin for the manufacture of a medicament for the treatment or prevention of osteoporosis.

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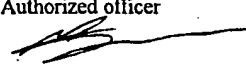
28. Use according to claim 22 wherein the daidzein or formononetin is administered from 1 to 6 times in a 24 hour period so as to give a daily dosage of about 5 mg to about 400 mg.
29. Use according to claim 27 wherein the isoflavone daidzein is combined with formononetin.
30. An agent for the treatment or prevention of menopausal symptoms or osteoporosis which comprises formononetin, or an agent for the treatment or prevention of menopausal symptoms which comprises daidzein, optionally in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.
31. A method or composition according to claim 1 or 14, wherein the isoflavone daidzein is in the aglycone, glycoside, malonyl or acetyl form.



## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 98/00313

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int Cl <sup>6</sup> : A61K 031/35		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC : A61K, Search Terms as below		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU : IPC as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) <b>DERWENT WPAT; DAIDZEIN or DAIDZIN or FORMONONETIN or EQUOL or DEHYDROEQUOL CA (STN); MEDLINE (STN): or DESMETHYLANGOLENSIN</b>		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/23069 A (KELLY, G.E.) 25 November 1993 see whole document	1, 4-10, 12-14, 17-23, 25-31
X	WO 94/23716 A (TUFTS UNIVERSITY SCHOOL OF MEDICINE) 27 October 1994 see whole document	1, 4-10, 12-14, 17-23, 25-31
X	US 5, 569, 459 A (BIO-VIRUS RESEARCH INCORPORATED) 29 October 1996 see whole document	1, 4-10, 12, 14, 17-18, 22-23, 25, 27-28, 30-31
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>		
Date of the actual completion of the international search 21 May 1998		Date of mailing of the international search report <b>30 JUN 1998</b>
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer  <b>MICHAEL GRIEVE</b> Telephone No.: (02) 6283 2267

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 98/00313

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/04248 A (ENERGETICS, INC.) 5 February 1998 see page 4 lines 21-33, page 10 line 7 to page 11 line 28	1-2, 4-10, 12, 14-15, 17- 18, 22-23, 25, 27-28, 30-31
X	US 5,352,384 A (PROTEIN TECHNOLOGIES INTERNATIONAL INC.) 4 October 1994 see whole document	14-18, 30
X	Patent Abstract of Japan, C-722, page 98 JP 2-67218 A (NAGAKURA SEIYAKU KK) 7 March 1990 see whole document	14-18, 30
X	US 5, 523, 087 A (BIO-VIRUS RESEARCH INC) 4 June 1996 see whole document	14-15, 20, 30- 31
X	US 5, 516, 528 A (WAKE FOREST UNIVERSITY and PROTEIN TECHNOLOGIES INTERNATIONAL, INC.) 14 May 1996 see whole document	14-15, 20, 30
Y	Joannou G. et al., 'A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids' <i>J. Steroid Biochem. Molec. Biol.</i> 54 (3/4) (1995) 167-184 see especially page 182	1-5, 14-18, 22- 25, 27, 30
Y	Pradhan, S.N. (Ed.) "Pharmacology in Medicine: Principles and Practice" (SP Press International Inc., Maryland, U.S.A., 1986) see pages 466-467	1-5, 14-18, 22- 25, 27, 30
Y	Wintrobe, M.M. (Ed.) et. al. "Harrison's Principles of Internal Medicine, 7th. Ed." (McGraw-Hill Kogakusha Ltd., Tokyo, Japan, 1974) page 1968	1-5, 14-18, 22- 25, 27, 30
Y	Reynolds, J.E.F. (Ed.) "Martindale : The Extra Pharmacopocia, 13th Ed." (The Pharmaceutical Press, London, U.K., 1993) pages 856.1, 1060.1, 1169.1, 1170.1	1-5, 14-18, 22- 25, 27, 30
Y	Berkow, R. (Ed.) et. al. "The Merck Manual of Diagnosis and Therapy, 16th Ed." (Merck Research Laboratories, Rahway, U.S.A., 1992) pages 1357-1359, 1793-1795	1-5, 14-18, 22- 25, 27, 30
Y	Budavan, S. (Ed.) et. al. "The Merck Index, 11th Ed." (Merck & Co. Inc., Rahaway, U.S.A., 1989) page 663 Monograph no. 4157	1-5, 14-18, 22- 25, 27, 30

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.  
PCT/AU 98/00313

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	93/23069	AU	40525/93	EP	656786	NO	944435
		NZ	252051				
WO	94/23716	AU	66359/94	CA	2160371	EP	693927
		US	5498631				
WO	98/04248	US	5654011				
US	5352384	US	5320949				
US	5516528	AU	46976/96	CA	2210811	WO	96/21403
END OF ANNEX							